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Review

DISEASES AND CONDITIONS ASSOCIATED WITH ZINC DEFICIENCY. SERUM ZINC IN PREGNANT WOMEN WITH REPRODUCTIVE FAILURES AND SERUM ZINC IN CHILDREN WITH TYPE I DIABETES

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ABSTRACT
Apart from severe zinc deficiency in akrodermatitis enteropatika, clinical effects of more moderate zinc deficiency are diarrhea, pneumonia, behavioral problems, impaired memory, learning disability and neuronal atrophy, alopecia, growth retardation, gonadal hypofunction, abnormal pregnancy, susceptibility to infections, delayed wound healing, impaired glucose tolerance, and many others. These effects are obtained in clinical studies that give a positive response to zinc supplement therapy. In this aspect, our study is a brief overview of diseases and conditions where zinc deficiency is observed.
The second part of the study presents our results on the levels of serum zinc in women with reproductive failures and in children with type I diabetes. The results showed that serum zinc was in deficient levels. We recommend proper diet, including foods and fortifitsirani fortified multivitamin preparations with zinc.
In conclusion we consider that the addition of zinc to the diet could improve the current status of patients with zinc deficiency and could even lead to survival of children with various diseases and conditions.
Due to varied results for concentrations of zinc and other trace elements in blood serum as indicators of micronutrient status it is necessary to carry out more detailed studies to identify the reasons and mechanisms of the micronutrient deficiency in different disorders.

Key words: zinc deficiency, pregnancy - reproductive failures, Type 1 Diabetes Mellitus

CONSEQUENCES OF ZINC DEFICIENCY

In the first part of this article we present a brief review on common diseases and conditions where zinc deficiency is found, while in the second part we present our own studies on patients in relations with zinc deficiency.

Due to many biological functions of zinc and its distribution in almost all human tissues, there is a wide range of physiological signs of zinc deficiency. These signs vary depending on the severity of the deficiency. Clinical signs of apparent zinc deficiency are seen in acrodermatitis enteropathica, which is a rare autosomal recessive genetic disease with zinc malabsorption (1). Clinical manifestations of zinc deficiency may vary in different ages. In early childhood, a common symptom is diarrhea. Furthermore, zinc deficiency leads to behavioral problems, impaired memory, learning disability and neuronal atrophy (2, 3).

Dermatic problems are becoming more common as the child grows. Alopecia, growth retardation and recurrent infections are common in school-aged children. Chronic skin ulcers and recurrent infections are common among older people.

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These effects are obtained in clinical studies that give a positive response to zinc supplement therapy. Unlike akrodermatitis enteropatika where the signs indicate massive deficit, functional disability can occur with mild or moderate deficits.

It is believed that zinc deficiency is commonly seen in the following medical conditions:

Diarrhea:
Extensive studies of zinc supplements conducted in countries in Asia, Latin America and Africa showed 25% reduction in the incidence of diarrhea and reduction of the number of days with diarrhea (4). These effects are not age dependant and showed that zinc - supplements may be used preventively in areas where there is high risk of zinc deficiency. Zinc supplementation results in reductions in diarrhea and pneumonia mortality (5).

Respiratory infections:
Reducing the incidence of acute infections in lower respiratory tract in response to zinc supplementation was also found in large number of studies. Studies conducted in children in India, Jamaica, Peru and Vietnam showed 41% overall reduction in the incidence of pneumonia after zinc supplements (6).

Patients with pneumonia have been found to have lower blood zinc levels compared to uninfected children. Zinc supplements are used to treat pneumonia (7). Zinc adjunct therapy reduces case fatality in severe childhood pneumonia (8). Zinc supplementation to maintain normal serum zinc concentrations in the elderly may help reduce the incidence of pneumonia and associated morbidity the role of zinc supplementation in elderly nursing home residents for the prevention of infection and amelioration of immune response (9).Other studies have concluded that there is no significant difference in frequency or duration of respiratory tract infection between the zinc-supplemented and control group of pediatric patients who were not taking zinc supplements (10). Such conclusions are usually made in mild conditions and at lower doses of additives.

Malaria:
Zinc may reduce the incidence of diseases-related to Plasmodium falciparum infections. Studies show 32-38 percent reduction in physician visits due to malaria after given 70 mg of zinc twice weekly or 10 mg of zinc per day (11). Overall studies have shown that zinc can reduce the severity of infection; it is possible that it leads to fewer clinic visits, but not necessarily to reduce the number of infections. The enzyme with high affinity for zinc exhibits partial collapse of structure upon removal of the metal ion. Zinc reconstitution of apo Plasmodium falciparum Silent information regulator 2 (PfSir2) led to recovery of both structure and activity highlighting the reversibility of the process (12)

Mortality
There is contradictory data in the literature regarding reduction of child mortality due to the effects of zinc supplements (13, 14). Most researchers in recent years, however, proved that prevention of infant mortality in diarrhea, pneumonia, malaria is a result of adding Oral zinc (15, 16).

Growth and Development
Zinc affects growth and development, even though the mechanisms of its action have not been thoroughly explored. This effect is observed at different life stages and under different individual physiological data.

Infants with low birth weight: Infants born with low gestational age weight are at high risk of zinc deficiency. Studies indicated weight and height gain in infants with low birth weight, receiving a daily supplement of 10 mg zinc for 60 days. (17). Infants in this type of studies have shown growth rate improvement (5 mg elemental Zn per day (n = 45) or placebo (n = 45) until 6 month of age), but it is believed that more researches are needed in this area to confirm this fact (18).

Severely malnourished children and handicapped children: Children with severe malnutrition suffer from multiple nutritional deficiencies, including zinc deficiency. Studies (19) showed weight gain in severely malnourished children who received daily zinc supplements (10 mg / kg body weight) for 6 months. In an older study where severely malnourished children received 1.5-6 mg / kg

body weight of zinc supplements for 15 to 30 days at higher doses and more prolonged intake within 90 days of follow increased risk of severe complications is accounted(20). Clearly, studies are needed to establish the dose and timing of intake of zinc supplements for nutritional rehabilitation.

In the *immature children* mega analysis by Kenneth H Brown et al. - 33 randomized trials (21) showed that zinc supplementation leads to significant improvement in weight gain and improved linear growth. Here the positive responses of zinc supplements on growth of children with low initial weight or height.

Adolescents:
In this group zinc deficiency is observed in anemia, delayed sexual development, short stature, increased liver and spleen, skeletal anomalies in the state. These signs and physiological conditions were observed in the first cases of investigated human zinc deficiency (22). Addition of zinc (10 mg / day for 12 months) resulted in significant increase of height and weight, to improved sexual maturation, improvement in bone development (23). Zinc deficiency is also associated with impaired appetite and thus can lead to decreased food intake and reduced growth (24).

Significant reduction of the physical signs of anorexia occurs in chronically ill patients after zinc supplementation (25). First data regarding improvement the condition of children with infantile anorexia after taking products with iron, zinc and vitamin A is published (26). It is hard to determine whether anorexia and bulimia are result of deficiency of zinc and other micronutrients or deficiency is a consequence of these diseases (27), but the effect of zinc supplements on growth and appetite is obvious and should be considered that supplementation is necessary for those conditions.

Pregnancy:
The results of studies with zinc supplements conducted during pregnancy are inconsistent. In some studies no effect on the improvement of the growth of the unborn child by adding zinc are found - in others - that there is improved fetal growth (28, 29). One study showed a reduction of pregnancy induced hypertension by supplements of zinc (30). In other studies, zinc deficiency is not associated with blood pressure in pregnant women (31). Zinc, folic acid and iron are recommended by Schumacher, I. and Piso, B., 2012 for malnutrition, and bed rest for hypertension for pregnant women (32).

Possible reasons for these discrepancies are varying degrees of zinc deficiency and different times and doses of supplements. There are more studies showing beneficial effects of zinc supplements during pregnancy and lasting effect on children's development after birth (33). Better controlled studies in pregnant women are needed in countries where there is an increased risk of zinc deficiency and high incidence of newborns with low weight.

Changes in the elderly
Some of the degenerative changes associated with aging may in part be due to zinc deficiency. Those include decline in immune function (34), delayed wound healing (35) and certain changes in neurological and psychiatric function in the elderly (36).

In the retina zinc is found in high concentrations. As we age, zinc decreases and there is a hypothesis that it plays a role in the development of age-related macular degeneration (Age-related macular degeneration, AMD). Clinical studies have found out that the progression of AMD assuming zinc, vitamin C, vitamin E, beta carotene and copper can be slowed down. Not all studies have found that zinc supplements are helpful for people with early signs of macular degeneration, but in recent years positive estimates and effects dominate, so taking of zinc additives is justified and further research on the problem is justified as well (37, 38, 39. 40).

Diabetes (Diabetes Mellitus)
Moderate zinc deficiency is relatively often seen in people with diabetes. It is believed that increased loss of zinc that occurs in diabetics due to frequent urination, contributes to the reduction of zinc nutritional status (41).

Zinc has insulin-like effect, increases the expense of glucose inhibition of glycogen synthesis. (42). Low gastrointestinal absorption and high urinary excretion of zinc in patients with diabetes may explain hipotsintsemiyata

observed in diabetes. Hyperzincuria may be due to hyperglycemia than any specific effect of endogenous or exogenous insulin on renal tubules. Hyperglycemia is associated with active transport of zinc back into the tubule cells. (43, 44). Although zinc supplementation improves immune function in diabetic patients, zinc supplementation of 50 mg / day adversely affects blood sugar control in insulin-dependent (type 1) diabetes - a study of Cunningham JJ and co-authors (1994) (45). In a recent study, supplements of 30 mg / day of zinc for six months in patients with type 2 diabetes reduces oxidative stress, no significant negative impact on blood sugar control is observed (46). We believe that the influence of zinc on glucose metabolism has not yet been fully elucidated, thus requiring further research before they recommendations for higher doses of zinc in diabetic patients (47, 48).

HIV infection / AIDS

There is considerable interest in the role of trace elements in the prevention and treatment of infectious diseases among adults and children. (49). In the case of human immunodeficiency virus - HIV infection, several randomized trials have shown the benefits of micronutrient supplements, including reduced risk of disease progression, especially among people in the early stages of the disease (50). Although highly active antiretroviral therapy (HAART) leads to significantly improved health outcomes, the risk of opportunistic infections and mortality are still high (51). The question of whether micronutrient supplements have a role in the treatment of individuals already receiving HAART is currently an area of active research. Baum et all, 2010 (52) show the results of a randomized, controlled trial of zinc supplementation among HIV-infected adults in the United States. They examined the efficacy of daily supplementation with nutritional doses of zinc (12 mg of elemental zinc for women and 15 mg of elemental zinc for men) for 18 months among 231 HIV-infected patients who had plasma zinc levels <0.75 mcg / ml. Slowing the progression of HIV disease and prevention of HIV-related morbidity and mortality was observed. The authors found that after 18-month zinc supplementation decreased 4-times the risk of immunological failure, however, had no effect on viral load and mortality. Zinc supplementation also reduced the rate of diarrhea in more than half of the patients in the placebo group.

HIV also requires zinc and a higher zinc intake may stimulate progression of HIV infection. Therefore the role of zinc supplements in some studies is controversial. The addition of zinc had no significant effect on the immunological status in patients with AIDS because of small group size and relatively short period of research (53).

To assess whether micronutrient supplements are effective and safe in reducing mortality and morbidity in adults and children with HIV infection a major survey by Irlam, JH et al., 2010 was conducted (54). Selected randomized controlled trials compared the effects of micronutrient and vitamin supplements, and their combinations with other supplements, placebo or not they examined mortality, morbidity, outcome of pregnancy, immunological parameters and anthropometric measures in HIV- infected adults and children (54). These authors found that zinc supplements reduced diarrhea and had no adverse effect on disease progression in children in South Africa. No significant clinical benefits of zinc - supplement for pregnant women and the impact on persistent diarrhea in adults from Tanzania and Peru was found.

Multiple micronutrient supplements reduced morbidity and mortality in HIV-positive pregnant women and their offspring, and improve early child growth in other large randomized controlled trial in Africa, reported by Irlam, JH et all, 2010 (54). Additional studies are needed to determine whether they are valid findings. The study zinc supplements were safe in HIV-infected adults and children. There is benefit in HIV-infected children and uninfected adults and children with diarrhea (54).

There are several weaknesses in the study of Baum et all, 2010 (52) patients with zinc deficiency, used food doses, which reduces the possibility of a potential zinc toxicity and the possibility of progression of HIV infection due to higher intake of zinc. The duration of the test is reasonably long (18 months) to monitor the clinical effect. These findings strengthen the evidence supporting the use of zinc supplements in addition to antiretroviral treatment for HIV

infected patients and should be targeted towards supplementation for individuals with poor zinc levels.

By enhancing immune function, micronutrient supplements provided to individuals in early stages of HIV disease, delaying disease progression and could prolong the time before the implementation of HAART. As we move towards an era of more widespread use of HAART, particularly in developing countries, micronutrient supplements are also promising low as adjunctive therapy to reduce morbidity associated with infections disease as well as treatment to improve quality of life of people infected with HIV (55).

OUR OWN STUDIES

Material and methods

Samples of blood serum

The collected venous blood samples were placed into sterile, closed tubes. After two hours stay blood samples were centrifugated at 3500 rpm for 10 min and separated sera were put in closed plastic laboratory vessels and were kept at -18°C in the fridge. Blood serum samples, obtained from persons of different age, sex and health status, were used to determine the zinc levels by the below described analytical procedure. For determination of serum zinc in children - patients and controls of healthy children the following conditions were maintained: • samples were obtained on an empty stomach between 8 AM and 9.30 AM; • ethical approval was obtained from the institutional research ethics committee and the parents of all subjects gave written informed consent prior to enrolment in the study.

Analytical method for determination of zinc in blood serum

The essence of the analytical method and methodological changes

Method for determination of zinc in blood serum, adapted from Lampugnani et al (27), was proposed for this study (56).

The zinc quantity in blood serum was determined by spectrophotometric method after deproteinisation of samples. The chromogen used was 4-(2-pyridylazo) resorcinol sodium salt (PAR-Na). This method was modified to achieve maximum simplicity, better repeatability and sensitivity. The volume of blood serum samples and the number of reagents were reduced (from 7 solutions for reagents to 2).

The low limit of detection, provided by the method of Lampugnani et al (57), allowed five-fold decrease in the volume of studied blood serum. This is especially important in investigations on children. That modification decreases the number of measurements, volumes of the reagents and possibility for errors which provides better repeatability. At the same time all technical procedures and preparation of reagents are quite easy and fast. In our modification the standard solution of zinc oxide was replaced with zinc nitrate.

Reagents, laboratory equipment and the whole analytical procedure for determining zinc in serum are given by Angelova, et al., 2006 (56).

Zinc status

Zinc status was defined by serum zinc levels with international values between (58) 11.6 μmol l-1 and 23.0 μmol l-1. Serum/plasma zinc ratios are not useful in mild zinc deficiency when values are often within the normal range (59). For this reason serum zinc was measured in control groups of healthy children and in cases of significance, assessed by the use of the appropriate statistical method; zinc levels were considered as decreased or increased, although values were within the reference values. Zinc values below 10.71 μmol l-1 in morning samples of blood serum were accepted by World Health Organization as zinc deficiency (59).

Statistical analysis

Statistical analysis of results was made using Statgraphics Plus for Windows. All values were expressed as mean (X) ± standard deviation (SD). Student's t-test, Ratio, and one-way analysis of variance (ANOVA) was used. Correlation analysis was performed for selected data sets, and data were considered significant when p value was less than 0.05 (p).

Serum zinc in women with reproductive failures

The essential trace element zinc has a vital role in the conception and carrying of a fetus in humans. Its importance is determined by its participation as a cofactor in enzymes associated with the development of embryonic tissues and

cell migration. Zinc deficiency during pregnancy is associated with increased blood pressure in pregnant women with PTL. Therefore aim of our study was to examine serum zinc in women with reproductive failures - recurrent miscarriages, unsuccessful in vitro fertilization and / or stillbirths.

We determined the content of zinc in blood serum - 11.04 ± 4.73 μmol l-1 in14 women with reproductive failures aged 30.07 ± 8.63 D. In nine of them (64%) it was found to be scarce - 7.94 ± 1.07 μmol l-1 to normal - 17.5 ± 3.12 μmol l-1 **(Figure 1)** obtained for the control group (n = 15) women in the same age group - 28.52 ± 9.61 on

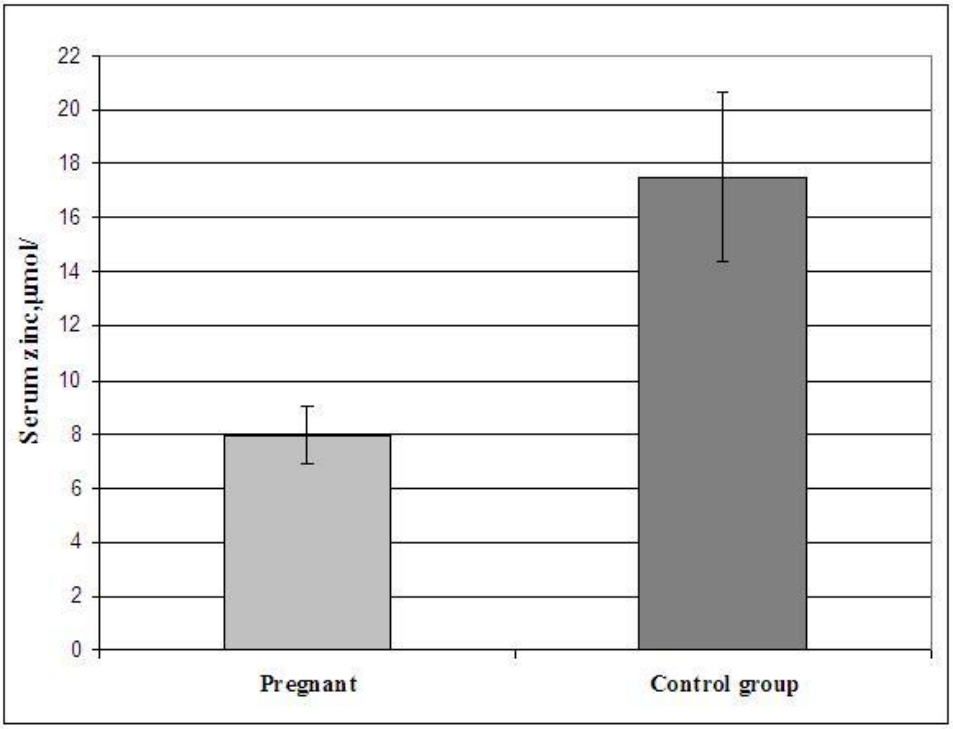

Figure 1. Zinc in blood serum in women with reproductive failures (n = 9) and control group (n = 15)- healthy women.

It was found that the need for zinc increases during pregnancy - 11-12 mg / day and pregnant women are at increased risk of zinc deficiency. Studies show that 82% of pregnant women worldwide have inadequate intake of zinc in their diet (60).

Poor nutritional status of zinc during pregnancy is associated with low birth weight, premature birth, birth complications and congenital anomalies (61).

Despite the severe effects observed, studies of zinc supplementation in the U.S. and developing countries give mixed results (62). Some of these studies have even been conducted in areas with pronounced zinc deficiency in populations. A review of 17 randomized controlled trials found that zinc supplementation during pregnancy is associated with a 14% decrease in preterm births. These results were observed primarily in women with low incomes (63, 64) and based on them zinc supplementation has not been accepted as an indicator of the health of the pregnant and / or the child.

It is known that iron supplementation not within the range of dietary iron levels may reduce the absorption of zinc and lead to zinc deficiency (65, 66).

Correlation analysis in our study (67) also showed such correlation between serum zinc, iron and other hematological parameters - hemoglobin and hematocrit.

Due to the fact the addition of iron is regulatory recommended during pregnancy - 38-65 mg / day of elemental iron for pregnant women who take higher doses - more than 60 mg / day iron as (68, 69), we also consider that for them in addition to food diversification and biofortification, supplemental multivitamin-mineral supplement that includes zinc may be considered as a way to improve child and maternal health.

The observed high proportion of women with reproductive failures with low levels of zinc in serum gives us reason to believe that there is a link between zinc deficiency during pregnancy and development of vascular and tissue changes leading to spontaneous pregnancy loss in our patients.

Zinc deficiency can be considered as a contributing ' Barker Effect" factor',which postulates that exposure in utero and postnatal environment is conducive to risk of autoimmune diseases such as asthma, diabetes, hypertension and coronary heart disease later in life. Thus, the effect of maternal zinc deficiency on birth defects may be deeper than seen at first glance.

In conclusion we can say that overall the evidence are in favor of the fact that pregnant women with zinc deficiency are at a greater risk for themselves and their offspring. Compelling evidence, however, in order to justify regulated addition of zinc during pregnancy like iron and folic acid, for example, are not yet available.

Serum zinc in children with Type 1 diabetes

This study provides data for serum zinc concentrations measured in a group of 29 children between 7 years and 16 year of age – 17 children with diabetes (Type I), and control group of 12 healthy children. The patients with diabetes were treated with a standard dose of human insulin obtained from Novo Nordisk Industry, Copenhagen, Denmark. The mean duration of diabetes was (3.64±3.22) year. All subjects were given 2 to 4 subcutaneous doses of insulin per day.

The following results were obtained for serum zinc: 10.29 ± 2.31 μmol l-1, C (min) - C (max), - 3.06 - 14.08 μmol l-1 for patients with diabetes and 18.28 ± 2.32 μmol l-1, C (min) - C (max) - 16.24 - 21.42 μmol l-1 for the control group. Test results of serum zinc in children with type 1 diabetes and control groups are presented in **Figure 2.**

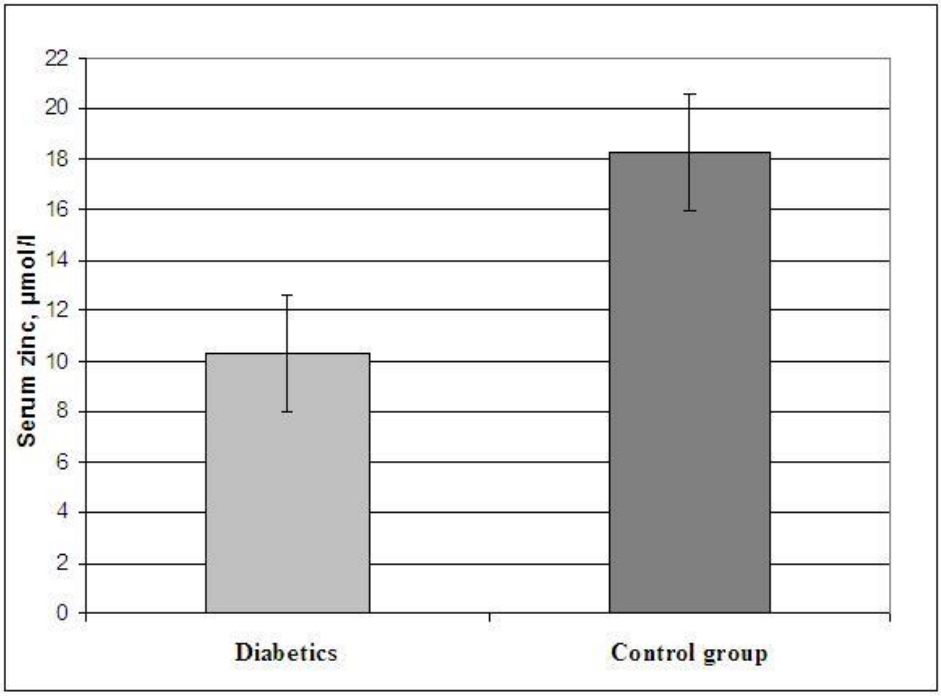

Figure 2. Serum zinc in children with Type 1 diabetes mellitus (n = 17) and control group (n = 12)

The serum zinc in diabetic children was lower than in controls ($p < 0.001$, 99.99 %) and in 53 % of the patients Zn (II) was below 10.71 μmol l^{-1} that indicated zinc deficiency. It is important to point out that diabetics have poor metabolic control of the disease – mean glycated haemoglobin value is 11.8 ±1.7 % (recommended value < 6.5 %). It is clear that the predominant effect on zinc homeostasis in diabetes is hypozincaemia, which may be the result of hyperzincuria or decreased gastrointestinal absorption of zinc or both (70, 71)**.**

Zinc is a cofactor for superoxide dismutase, which is an intracellular antioxidant enzyme. It has been suggested that as pancreatic beta cells have low antioxidative enzyme activities, zinc is also capable of modulating insulin action and it also improves hepatic binding of insulin. Abnormal zinc metabolism, therefore, could play a role in the pathogenesis of diabetes and its complications (72,73)**.**

Given the fact that zinc levels are lower than the recommended in 89.4% of children (74), Ortega, Rmet all 2012 (74) and we believe that increasing the intake of zinc can improve health and nutritional status of children with Type I diabetes, and thus contribute to reducing the problems of insulin resistance.

Furthermore, changes in lifestyle, the addition of zinc to the diet of diabetic obese children, can be considered (75, 76, and 77) as a useful and safe adjunctive therapy to improve risk factors for cardiovascular system associated with both insulin resistance and childhood obesity. In conclusion we could state that the available evidence suggests that zinc deficiency plays the above role in the development of diabetes as well as in the distribution of cardiovascular disease.

CONCLUSION

Zinc is an essential nutrient and plays an important role in child growth, immune function and resistance to infection in children and adults. Zinc deficiency puts children in many low-income countries at increased risk for illness and death from infectious diseases. Zinc deficiency is a problem for public health and nutrition. It affects a large number of children, women and adults all around the world. Zinc affects multiple aspects of the immune system - from skin to gene regulation in lymphocytes. Zinc is used in preventive studies with zinc supplementation. It is proved that it has a significant impact on the incidence of acute infections of the lower respiratory tract. Zinc is used for treatment of pneumonia, common cold and respiratory infections. Zinc supplements can reduce the frequency of clinical episodes of malaria in children. Sufficient quantities of zinc are essential for maintaining immune function and HIV-infected individuals are particularly sensitive to zinc deficiency. Reduction of serum zinc levels is associated with more advanced state of the disease and increased mortality in patients with HIV. The addition of zinc is commonly used in wound healing. Zinc deficiency plays a role in the development of diabetes as well as in the distribution of cardiovascular diseases. All these effects were observed in clinical studies that give a positive response to zinc supplement therapy. These conclusions were made by us by conducting a brief overview of common diseases and conditions where zinc deficiency is established.

The results obtained from our own studies on women with reproductive failures and in children with type I diabetes showed deficiency in sera zinc levels. Based on these our findings we consider that to prevent from zinc deficiency and its consequences a proper diet, including fortified food and multivitamin drugs with zinc could decrease the risk from possible complications and also would improve the state of the patients in both studied groups. On account of the varied results obtained about concentrations of zinc and other trace elements in blood serum as indicators of micronutrient status, more detailed studies have to be conducted to evaluate the reasons and mechanisms of the micronutrient deficiency, in different disorders.

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